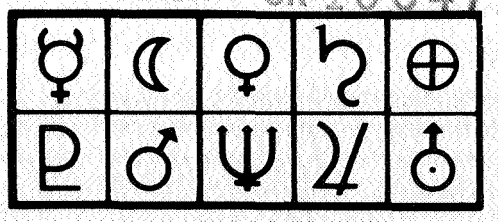

PLANETARY QUARANTINE

QR 14
September 1969

SANDIA LABORATORIES QUARTERLY REPORT - PLANETARY QUARANTINE PROGRAM

Planetary Quarantine Department 1740

A.E.C.

SANDIA LABORATORIES

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for

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Planetary Quarantine Department

Sandia Laboratories, Albuquerque, New Mexico

September 1969

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Kinetic Modeling of Microbial Inactivation

A. Description. This activity has as its aim the development of a sterilization model that is physically based and is consistent with all known data related to microbial survivors in dry heat.

B. Progress. Preliminary indications that available water affects microbial inactivation rate through its effect upon the activation entropy of the biologically inactivating reactions were reported on last quarter (QR 13). In that report, it was pointed out that experimental variations in the entropy of adsorption of some macromolecules as a function of available water had a characteristic shape compatible with the change in entropy predicted by the kinetic model based upon survivor data reported in Ecology and Thermal Inactivation of Microbes in and on Interplanetary Space Vehicle Components, Fourteenth Quarterly Report of Progress, USPHS, Cincinnati, March 1969. The data in this latter report were in the form of D-values and, thus, not ideally suited for use with the kinetic model in view of the extreme sensitivity of survivors to entropy changes in some ranges of available water. Dr. J. E. Campbell at Cincinnati kindly provided us with their raw data this past quarter and studies to challenge the model with this data were undertaken.

Figure 1 shows the temperatures and water levels at which survivor data were available. The data were obtained for spores embedded in epoxy in each case.

a_w T	125°C	135°C	140°C
0	X		
0.03	X	X	X
0.05	X	X	X
0.07	X	X	X
0.10	X		
0.20	X		X
0.30		X	
0.40	X		X
0.60	X		X
0.80	X	X	X
0.90	X	X	X
1.00		X	X

Figure 1 - Data Ranges

The data at these temperatures and water levels were all of the slightly convex form shown in Figure 2.

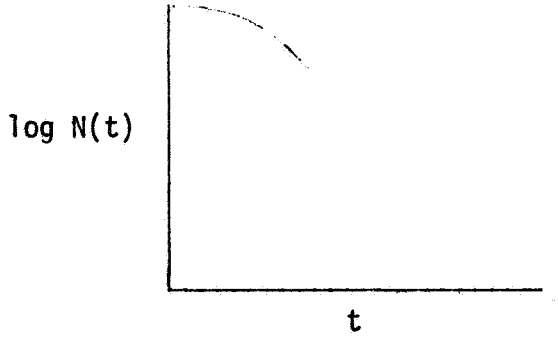

Figure 2

The subportion of the kinetic model best representing data of this form corresponded to a first order reaction

$$A \rightarrow D$$

with two molecules of A to be inactivated. Using this submodel with the reaction rate

$$k = \frac{KT}{h} e^{-(\Delta H^{\ddagger} - T\ \Delta S^{\ddagger})/RT},$$

the activation enthalpy $\Delta H^{\ddagger}$ was found to be essentially 26.975 K cal/mole for <u>each</u> water level at which data for all three temperatures were available. This activation enthalpy value was then assumed to be that associated with the particular spore crop and general environment (epoxy) used by PHS Cincinnati in obtaining the data. That is, k was assumed to be of the form

$$k = \frac{KT}{h} e^{-(26.975 - \Delta S^{\ddagger} T)/RT}$$

wherein $\Delta S^{\ddagger}$ is the only remaining variable at fixed temperature, T. Using the above submodel of the kinetic model with k having the above form, the model was used to generate an activation entropy curve, $\Delta S^{\ddagger}$, as a function of water at 125°C. This is shown in Figure 3.

Thus, in theory, $\Delta H^{\ddagger}$ is determined by the general experimental situation and nature of the spores while $\Delta S^{\ddagger}$ is a function of these

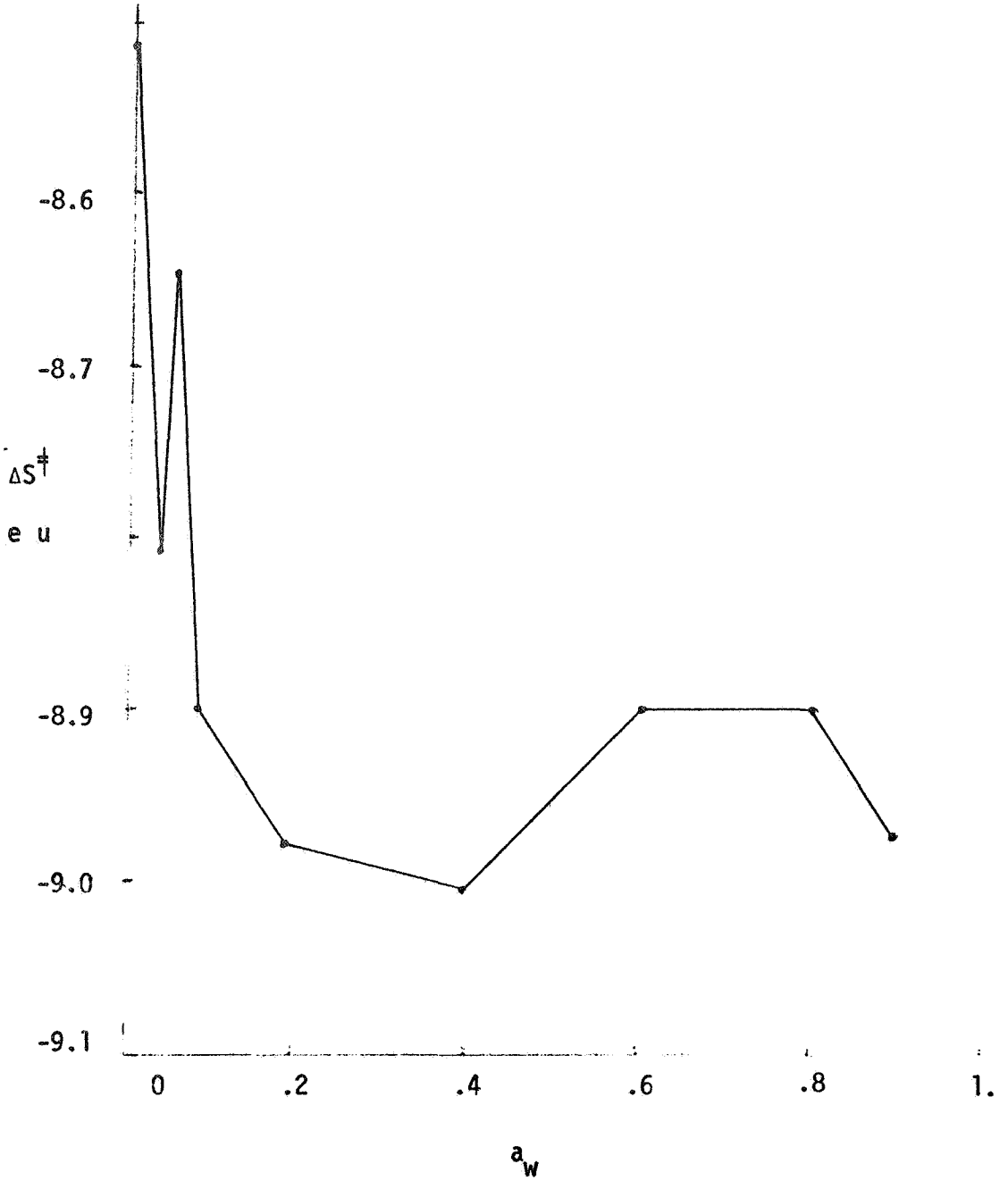

FIGURE 3 - CALCULATED DEPENDENCE OF ACTIVATION ENTROPY $\Delta S^{\ddagger}$ ON AVAILABLE WATER USING DATA AT 125°C.

things and the available water. Since both appear independent of temperature, it should be possible to predict the survivor behavior at temperatures other than 125°C (used in generating Figure 3) and various water levels using the kinetic model, $\Delta H^{\ddagger}$ at 26.975 K cal/mole and $\Delta S^{\ddagger}$ as it occurs on Figure 3. Such attempted predictions provide a check on the model itself and on the way in which water is assumed to effect survivor rates through its influence on activation entropy, $\Delta S^{\ddagger}$.

Two of the predictions at other temperatures and water levels are shown in Figures 4 and 5. The data is that obtained from J.E.Campbell and is shown as dots. The continuous curve is that predicted by the kinetic model. In general the predictions were accurate to the degree demonstrated in these figures. At times, however, comparisons of the type shown in Figure 6 were encountered. Here, the prediction at 140°C was quite accurate (shown also in Figure 4), but that at 135°C was 3/4 of a log off at 6 hours. This deviation occurs in a region of a_w where a very slight change in a_w greatly effects the survivor curve (as may be seen from Figure 3). Thus, an inaccuracy of only several thousanths a_w in measurement would be sufficient to account for the deviation of the prediction at 135°C in Figure 6. In general, when they occurred, difficulties in predicting survivor curves occurred in regions on Figure 3 where the slope of the curve $\Delta S^{\ddagger}$ versus a_w is steep. This is not unexpected because of the extreme sensitivities to slight variations in a_w.

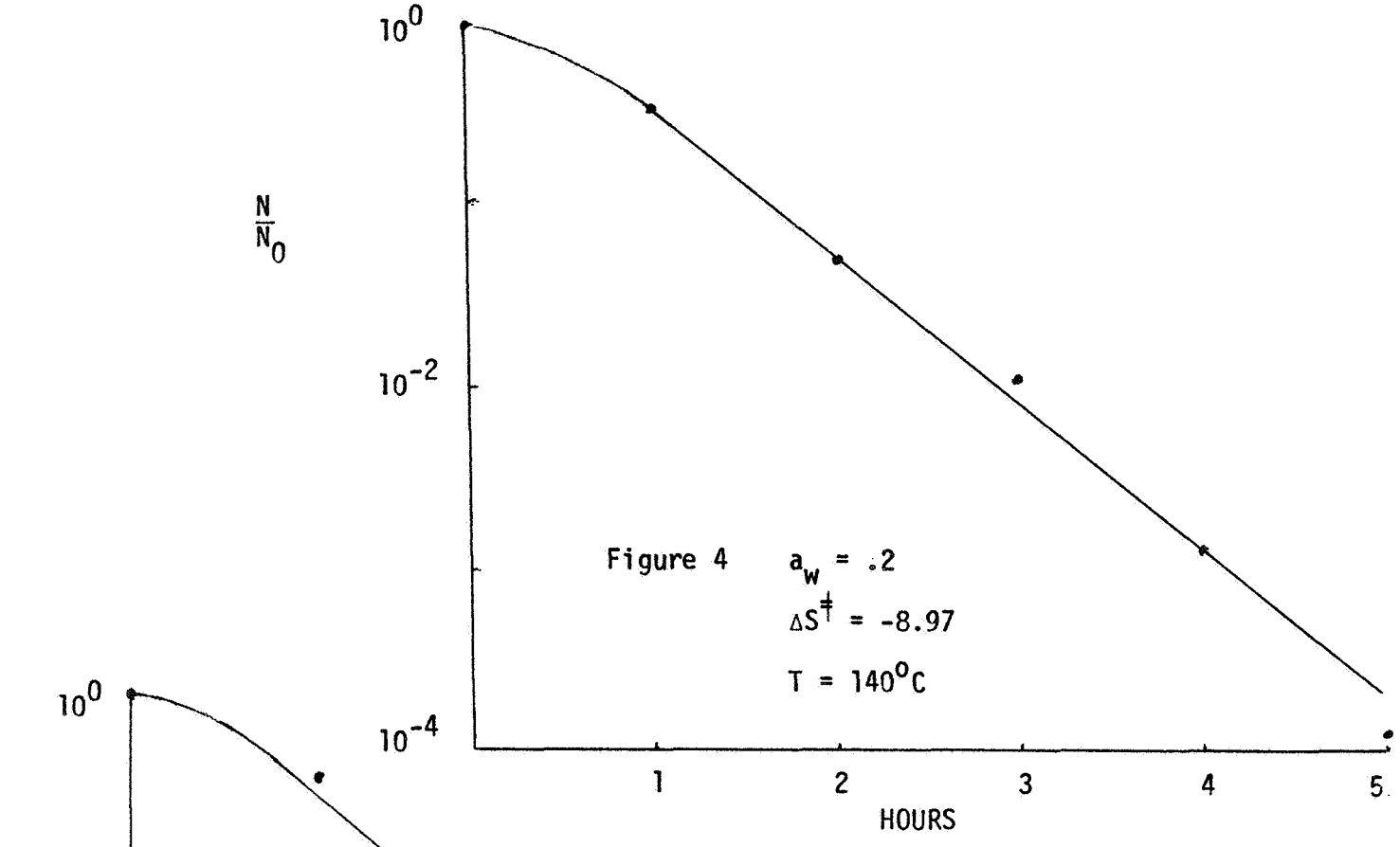

Figure 4 $a_w = .2$

$\Delta S^{\ddagger} = -8.97$

$T = 140^oC$

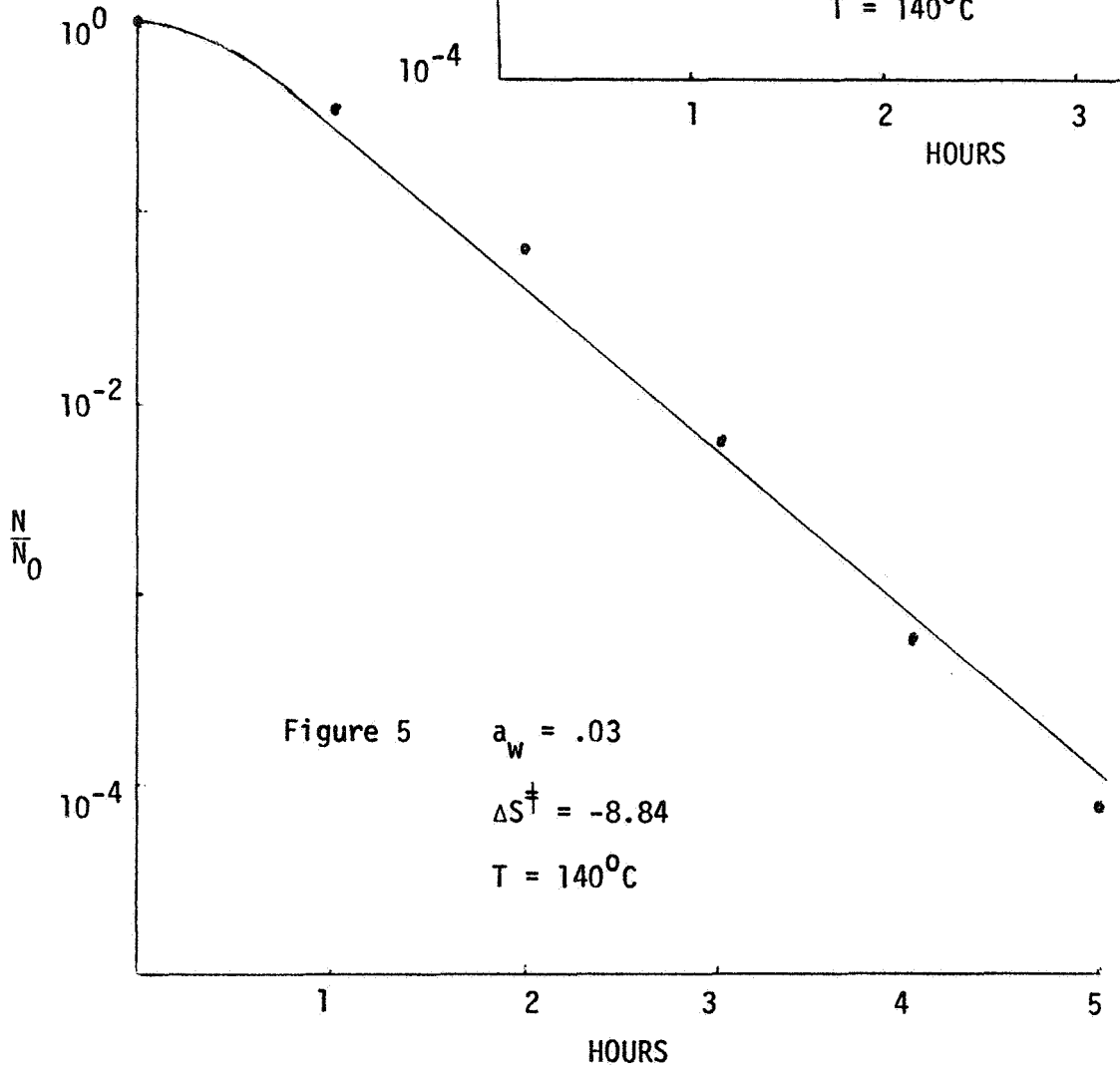

Figure 5 $a_w = .03$

$\Delta S^{\ddagger} = -8.84$

$T = 140^oC$

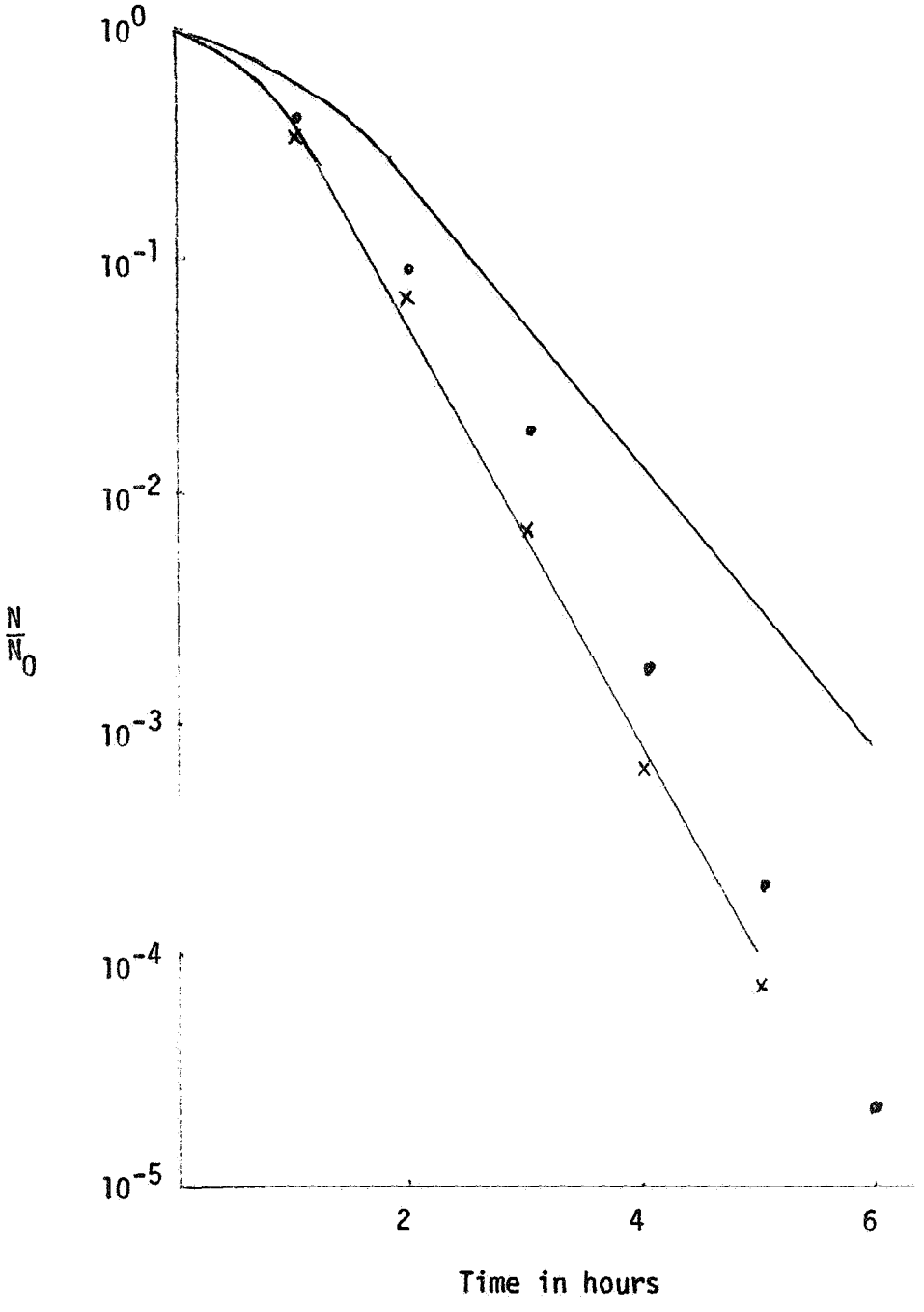

Figure 6

$\Delta S = -8.84$ $a_w = .03$

• = 135°C

X = 140°C

Lunar Planetary Quarantine Study and Information System

A. Description. The objective of this activity was to develop a management information system whose outputs provide the information needed by the Planetary Quarantine Officer in fulfilling his responsibilities as they appear in NASA Policy Documents 8020.7 and 8020.8A.

B. Progress. This activity was completed during the past quarter. The overall activity may be divided into the following four gross categories:

1. Problem analysis,
2. System design
3. System programming, and
4. System checkout.

For purposes of problem analysis, the gross lunar responsibilities of the Planetary Quarantine Officer were assumed to be:

i. certification that the biocontamination levels of U.S. unmanned lunar probes do not exceed a specified maximum,

ii. certification that the biocontamination levels of U.S. manned missions have been held to a practical minimum consistent with achieving the major objectives of the missions,

iii. maintenance of a file of qualitative spacecraft bioburden information in which "types" of organisms found on manned missions prior to launch are stored by "type" and the portion of the mission on which they were found, and

iv. maintenance of an inventory of probable lunar biocontamination levels as a function of lunar coordinates and time.

Based on these responsibilities, an analysis was performed to indicate both the types and amounts of data and flight information needed to fulfill them.

Following this, a system was designed which would store and analyze the needed data and information to yield information consistent with the assumed responsibilities. This design appears in "An Interactive Computer Information System for Planetary Quarantine for Lunar Programs", Sandia Laboratories Research Report, SC-RR-68-545, July 1968. A gross flow chart of this system is shown in Figure 1.

Models required in this system for data processing are included as subroutines and have been reported on separately. In particular, data related to quantitative bioburdens at launch are extrapolated using the model developed in "The Determination of Quantitative Microbial Sampling Requirements for Apollo Modules", Sandia Laboratories Research Report SC-RR-69-23, January 1969. A sample system quantitative output from DAST is shown in Figure 2. Here, the numbers represent estimates of the expected bioburden of Command Module 0171N as of July 16, 1969 (196 days beyond January 1, 1969 - the system reference date) given as mean number per square foot of surface area. Figure 3 shows the same estimate for the total Command Module. Based upon the model referenced above, 90% confidence intervals for these figures is also shown. In both cases, the system is capable of projecting the expected burden <u>in time</u> when certain types of environmental data are available. Since these data are not currently

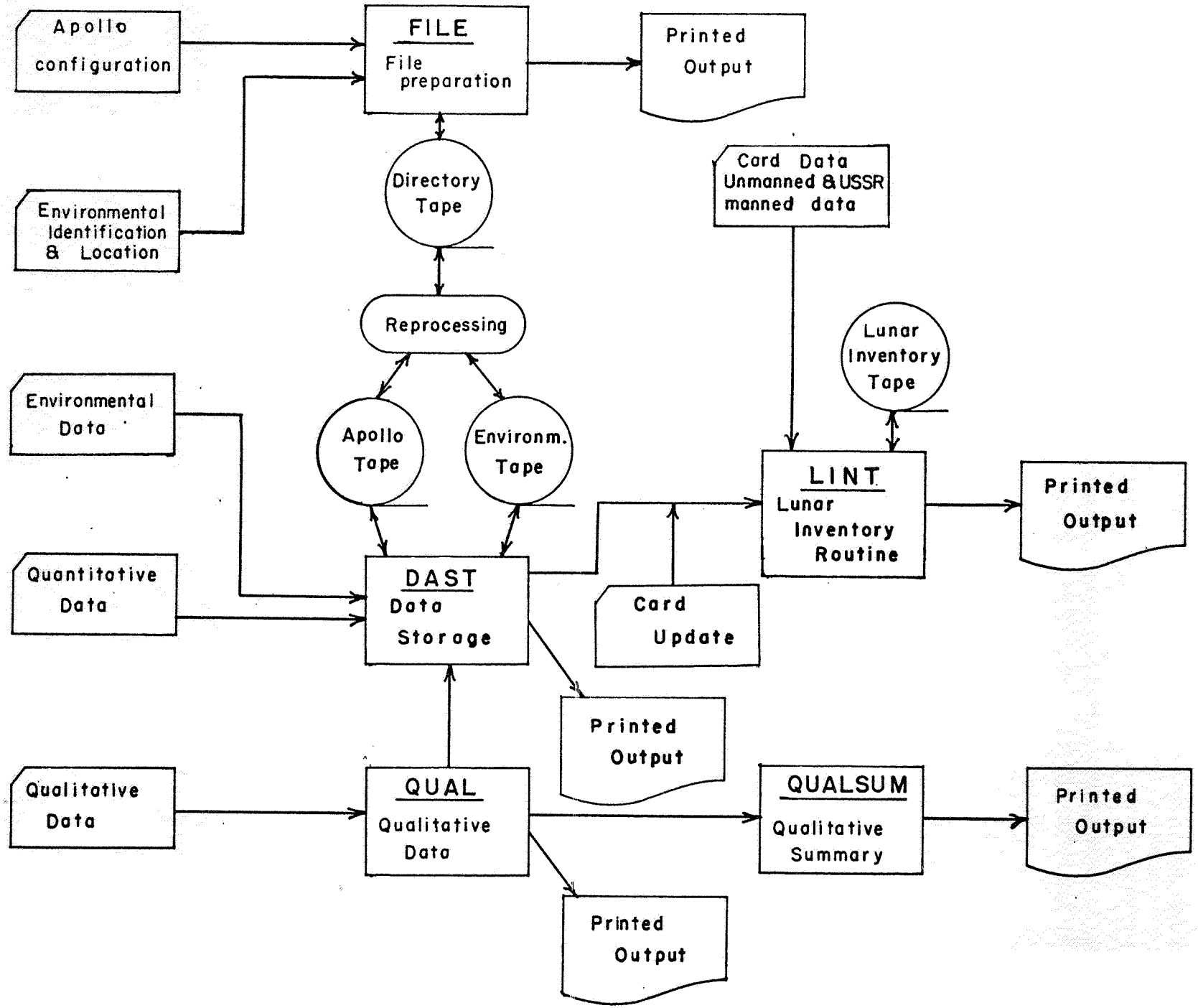

FIGURE 1 - GROSS SYSTEM FLOWCHART

QUANTITATIVE OUTPUT

COUNTS PER SQ.FT.

MODULE = CM0107IN
SAMPLING DATE = 196

MEAN NUMBER

	SAMPLE DATE	LAUNCH DATE	IMPACT DATE
AER.VEG.	9.7812E 03	***	***
ANER.VEG.	4.0248E 03	***	***
AER.S.F.	2.9640E 02	***	***
ANER.S.F.	4.6800E 01	***	***
0.90 CONF.LIMIT			
AER.VEG.	9.7910E 03	***	***
ANER.VEG.	4.0311E 03	***	***
AER.S.F.	2.9810E 02	***	***
ANER.S.F.	4.7477E 01	***	***

FIGURE 2 - BIOBURDEN PER UNIT AREA.

QUANTITATIVE OUTPUT

MODULE = CM0107IN.
SAMPLING DATE = 196

MEAN NUMBER

	SAMPLE DATE	LAUNCH DATE	IMPACT DATE
AER.VEG.	5.3699E 06	***	***
ANER.VEG.	2.2096E 06	***	***
AER.S.F.	1.6272E 05	***	***
ANER.S.F.	2.5693E 04	***	***
0.90 CONF.LIMIT			
AER.VEG.	5.3763E 06	***	***
ANER.VEG.	2.2131E 06	***	***
AER.S.F.	1.6366E 05	***	***
ANER.S.F.	2.6065E 04	***	***

FIGURE 3 - TOTAL BIOBURDEN

available, this column and subsequent projections to the lunar surface, are left blank.

While DAST has some limited qualitative information output relating to organisms identified prior to launch (see Figure 4), the bulk of the qualitative information about a manned lunar mission is processed through QUAL and QUALSUM (ref. Figure 1). The probability that any "type" of organism actually on the craft has been identified is calculated using a model developed in "A Model for the Quantification of the Qualitative Sampling Problem", Sandia Laboratories Research Report", SC-RR-69-310, May 1969.

The subroutine QUAL stores (and prints out) qualitative data as it is entered by the PHS personnel at Cape Kennedy as a result of "typing" tests performed both there and in Cincinnati. Figure 5 shows a representative QUAL output. The B09-- refers to colony number, 190 the date of sample, the next two to module identification and sample number (not now used) and, for example, Staph.Group2, the assigned "type" of organism. The material in the printout below shows the outcomes of any of 64 possible tests that were run on the colony B09--.(These tests are described in "Identification Schemes for Microorganisms Isolated from Apollo Spacecraft", USPHS, NCDC, Phoenix).

QUALSUM performs certain analyses on the data stored in QUAL. Generally, it lists organisms by "type" and "treatment" found on various modules. Figures 6 & 7 show a sample of this output for the Lunar Module Ascent Stage (LAE Module). The "Observed" column

Qualitative Output

Module = _____

Sampling Date = _____

Sample Date	Launch Date	Impact Date
_____	_____	_____

(lists of organisms; room for 100)

Probability all have been identified = .xx

FIGURE 4 - QUALITATIVE OUTPUT FROM DAST

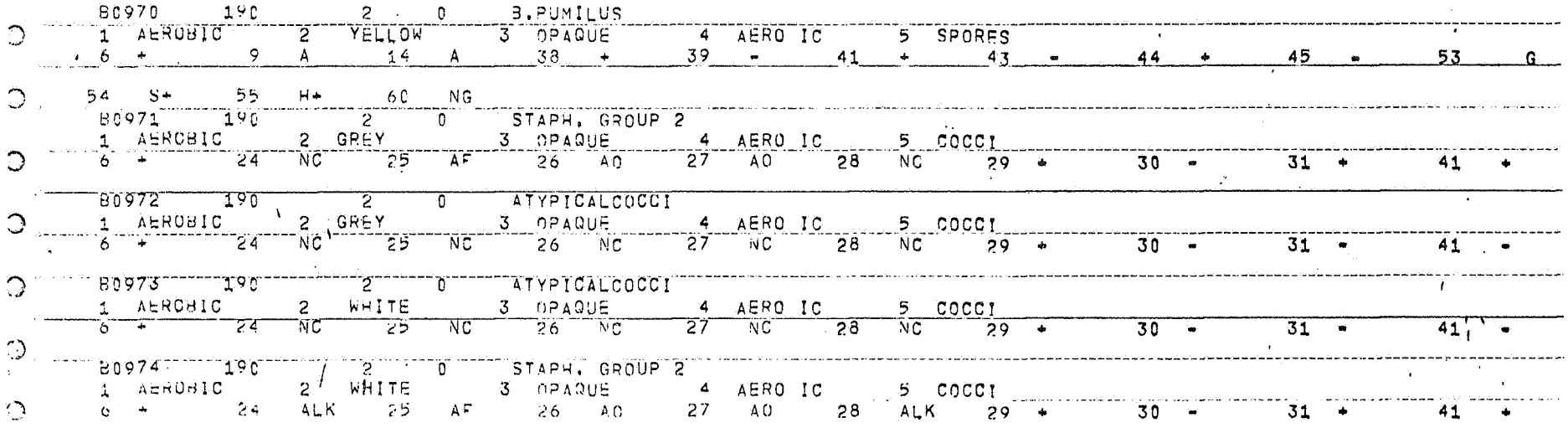

FIGURE 5 - SAMPLE QUAL OUTPUT

PERCENTAGES ON MODULE LAE FROM 28 COLONIES IDENTIFIED ON THIS MODULE BY SAMPLE TREATMENT BLD AGAR AEROBIC

KEY	ORGANISM	NUMBER	PERCENT	KEY	ORGANISM	NUMBER	PERCENT	KEY	ORGANISM	NUMBER	PERCENT
2	STAPH. GROUP 2	1	3.571	4	STAPH. GROUP 4	3	10.714	5	STAPH. GROUP 5	1	3.571
7	MIROC.SUB.GRP.1	6	21.429	8	MIROC.SUB.GRP.2	1	3.571	9	MIROC.SUB.GRP.3	1	3.571
13	MIROC.SUB.GRP.7	4	14.286	27	B.LENTUS	1	3.571	37	AER.NO S.F.G.P.R	5	17.857
91	MOLDS	2	7.143	95	NOGRO DIFF MEDIA	3	10.714				

PERCENTAGES ON MODULE LAE FROM 33 COLONIES IDENTIFIED ON THIS MODULE BY SAMPLE TREATMENT BLD AGAR ANAEROB

KEY	ORGANISM	NUMBER	PERCENT	KEY	ORGANISM	NUMBER	PERCENT	KEY	ORGANISM	NUMBER	PERCENT
1	STAPH. GROUP 1	1	3.030	2	STAPH. GROUP 2	4	12.121	4	STAPH. GROUP 4	5	15.152
5	STAPH. GROUP 5	2	6.061	6	STAPH. GROUP 6	2	6.061	7	MIROC.SUB.GRP.1	4	12.121
8	MIROC.SUB.GRP.2	4	12.121	9	MIROC.SUB.GRP.3	2	6.061	13	MIROC.SUB.GRP.7	3	9.091
25	B.FIRMUS	2	6.061	37	AER.NO S.F.G.P.R	2	6.061	92	ATYPICALCOCCI	1	3.030
95	NOGRO DIFF MEDIA	1	3.030								

FIGURE 6 - SAMPLE QUALSUM OUTPUT OF "TYPES" AND THEIR FREQUENCY

COLONY IDENTIFICATION CATALOGUE FOR LAE MODULE   CRAFT B

```
14  MIROC.SUB.GRP.8       1  1621,22  ,   ,   ,   ,   5,5555,211 ,   ,   ,1   ,   ,   ,   ,   ,
24  B,COAGULANS           1  3931,12  ,3  , 3 ,   ,   ,   ,   ,   , 12 ,2 11,1   ,   ,22  ,  2,
25  B,FIRMUS              1  6931,12  ,3  , 3 ,   ,   ,   ,   ,   , 21 ,2 10,2   ,   ,22  ,  1,
                          1  6921,42  ,3  , 1 ,   ,   ,   ,   ,   , 22 ,1 11,1   ,   ,22  ,  1,
27  B,LENTUS              1  1921,12  ,3  , 1 ,   ,   ,   ,   ,   , 11 ,0 10,1   ,   ,22  ,  2,
                          1  5821,42  ,3  , 1 ,   ,   ,   ,   ,   , 11 ,1 11,1   ,   ,22  ,  1,
28  B,LICHENIFORMIS       1  4821,12  ,3  , 1 ,   ,   ,   ,   ,   , 22 ,2 20,2   ,   ,22  ,  1,
29  B,MACERANS            1  3911,32  ,3  , 3 ,   ,   ,   ,   ,   , 21 ,1 11,2   ,   ,222 ,  1,
30  B,MEGATERIUM          1  1931,12  ,3  , 3 ,   ,   ,   ,   ,   , 22 ,1 11,1   ,   ,211 ,  1,
31  B,PATHOTHENICUS       1  1921,12  ,3  , 1 ,   ,   ,   ,   ,   , 21 ,2 10,2   ,   ,22  ,  2,
                          1  2931,12  ,3  , 1 ,   ,   ,   ,   ,   , 21 ,2 11,2   ,   ,22  ,  2,
                          1  2931,12  ,3  , 3 ,   ,   ,   ,   ,   , 22 ,1 11,2   ,   ,212 ,  2,
32  B,POLYMYXA            1  3921,32  ,3  , 3 ,   ,   ,   ,   ,   , 21 ,2 11,2   ,   ,222 ,  1,
34  B,PUMILUS             1  1921,32  ,3  , 3 ,   ,   ,   ,   ,   , 21 ,2 12,1   ,   ,222 ,  1,
37  AER.NO S.F.G.P.R      1  1411,12  ,2  ,   ,   ,   ,   ,2  ,1    , 11 , 1 ,1111,1  1,  ,  ,
                          1  1411,12  ,1  ,   ,   ,   ,   ,   ,1131,122 ,111 ,1311,1  1,  ,  ,
                          1  1911,12  ,1  ,   ,   ,   ,   ,2  ,1    , 22 ,111 ,1311,1  1,  ,  ,
                          1  5411,2   ,3  ,   ,   ,   ,   ,2  ,1131,111 ,221 ,1221,1  1,  ,  ,
                          1  5921,12  ,3  ,   ,   ,   ,   ,2  ,1131,111 ,221 ,1221,1  1,  ,  ,
                          1  5821,12  ,3  ,   ,   ,   ,   ,2  ,1131,111 ,111 ,2211,1  2,  ,  ,
                          1  5921,12  ,3  ,   ,   ,   ,   ,2  ,1131,111 ,221 ,1221,1  2,  ,  ,
                          1  1411,12  ,1  ,   ,   ,   ,   ,2  ,1    , 11 ,  1 ,1111,1  2,  ,  ,
                          1  1221,12  ,3  ,   ,   ,   ,   ,2  ,2111,111 ,211 ,2212,1  1,  ,  ,
                          1  141 ,12  ,1  ,   ,   ,   ,   ,1  ,1    , 22 ,111 ,2211,1   ,  ,  ,
                          1  142 ,12  ,3  ,   ,   ,   ,   ,2  ,1    , 21 ,111 ,1111,1  1,  ,  ,
                          1  141 ,12  ,3  ,   ,   ,   ,   ,2  ,3132,122 ,121 ,2221,1  1,  ,  ,
                          1  181 ,12  ,3  ,   ,   ,   ,   ,2  ,1    , 22 ,111 ,1211,1  1,  ,  ,
                          1  281 ,12  ,1  ,   ,   ,   ,   ,2  ,1    , 11 ,  1 ,1111,1  2,  ,  ,
                          1  242 ,12  ,3  ,   ,   ,   ,   ,2  ,2111,222 ,221 ,2221,1  1,  ,  ,
                          1  1931,12  ,3  ,   ,   ,   ,   ,2  ,1131,111 ,001 ,1111,1  1,1  ,  ,
                          1  1911,42  ,3  ,   ,   ,   ,   ,2  ,1131,1111,1 1 ,20  ,   1,1  ,  ,
                          1  1821,12  ,3  ,   ,   ,   ,   ,1  ,1131,122 ,001 ,1111,1  1,1  ,  ,
```

FIGURE 7 - SAMPLE QUALSUM OUTPUT OF "TYPES" AND "TYPING" SEQUENCE

of Figure 7 refers to the number of times a given "Organism" was identified and found to have the test sequence shown in the subsequent 64 columns.

The outputs of DAST, QUAL and QUALSUM seem sufficient to adequately meet responsibilities (ii) and (iii) and any future missions in (i) assumed earlier. The Lunar Inventory Routine (LINT) is designed to meet the remaining responsibilities.

Several examples of information output from LINT are shown in Figure 8. The lunar coordinates are shown as degrees East (+) or West (-) and degrees North (+) or South (-). The date, again, is days prior to (-) or subsequent to (+) January 1, 1969. The high "Total Burden" figures result from the assumption that hard impacts may yield a total spacecraft burden buried at sufficient depth to maintain its viability. The energies involved in impact are sufficiently high to make fragment burial possible, and lacking information about subsurface lunar temperatures, this conservative assumption was made. The models developed in Sandia Laboratories Monograph SC-M-68-539, "The Chances of Retrieval of Viable Microorganisms Deposited on the Moon by Unmanned Lunar Probes", are used extensively in LINT to obtain the output shown in Figure 8. A document completely describing the calculations made in LINT is being prepared. The Lunar Inventory Tape stores information on all missions impacted prior to the date such information is required.

The complete system was checked out on PHS Apollo 10 and 11 data and has been turned over to the PHS Cape personnel for future operation.

```
AT COORDINATES      -11.100       4.100     DATE  -800.50
DENSITY OF VIABLE VEGETATIVE  MICROBES IS LESS THAN  2.55397E-05 PER SQUARE METER
DENSITY OF VIABLE SPOREFORMER MICROBES IS LESS THAN  3.08723E-05 PER SQUARE METER
PROBABILITY OF CONTAMINATION OF A ONE SQUARE METER SAMPLE
       BY VEGETATIVE MICROBES          2.55394E-05
       BY SPOREFORMER MICROBES         3.08719E-05
       BY ANY VIABLE MICROBE           5.64105E-05

AT COORDINATES      -11.100       4.100     DATE  -799.50
DENSITY OF VIABLE VEGETATIVE  MICROBES IS LESS THAN  2.55400E-05 PER SQUARE METER
DENSITY OF VIABLE SPOREFORMER MICROBES IS LESS THAN  6.34684E-05 PER SQUARE METER
PROBABILITY OF CONTAMINATION OF A ONE SQUARE METER SAMPLE
       BY VEGETATIVE MICROBES          2.55397E-05
       BY SPOREFORMER MICROBES         6.34664E-05
       BY ANY VIABLE MICROBE           8.90045E-05
```

(a) Density Expected 150 Meters from Surveyor II Impact Site 1/2 Day Before and After Impact on September 23, 1966.

```
AT COORDINATES      -23.300      -2.900     DATE   318.00
DENSITY OF VIABLE VEGETATIVE  MICROBES IS LESS THAN  3.47883E-05 PER SQUARE METER
DENSITY OF VIABLE SPOREFORMER MICROBES IS LESS THAN  4.49262E-05 PER SQUARE METER
PROBABILITY OF CONTAMINATION OF A ONE SQUARE METER SAMPLE
       BY VEGETATIVE MICROBES          3.47877E-05
       BY SPOREFORMER MICROBES         4.49252E-05
       BY ANY VIABLE MICROBE           7.97114E-05
```

(b) Expected Densities at Anticpated Apollo 12 Landing Site, November 14, 1969.

FIGURE 8 - SAMPLE LINT OUTPUT

Distribution of Organic Material on the Lunar Surface

A. Description. A recent proposal to impact the Lunar Module Ascent Stage directly after post-touchdown rendevous rather than place this module in lunar orbit raised some concern about the effect on the recovery of organic material in subsequent lunar samples. Using the lunar bioburden distribution models developed in Sandia Laboratories Monograph SC-M-68-539, "The Chances of Retrieval of Viable Microorganisms Deposited on the Moon by Unmanned Lunar Probes", a rough conservative estimate was made of organic contamination densities on the lunar surface resulting from a LM ascent stage impact.

B. Progress. The model used for this estimate is the version of the crater debris model, cited above, that is currently operational in the lunar information system described elsewhere in this quarterly report. Crater debris, rather than spacecraft fragmentation, was chosen as the vehicle by which organic material is disseminated about the point of impact because crater debris density is greater than fragment density beyond a few kilometers. It was hoped that this choice was conservative.

Using a spacecraft weight of 5550 pounds and impact velocity of 5508 ft/sec the expected density of crater material at various distances from the point of impact is shown in Figure 1.

distance (kilometers)	crater debris density (gm/one-hundred sq.centimeters)
10	1.28×10^{-4}
20	2.26×10^{-5}
30	8.20×10^{-6}
40	4.00×10^{-6}
50	2.29×10^{-6}
60	1.45×10^{-6}
70	9.89×10^{-7}
80	7.08×10^{-7}
90	5.27×10^{-7}
100	4.06×10^{-7}

Figure 1 - Density of Crater Debris at Various Distance from Impact Point

The total amount of crater debris ejected was calculated to be approximately

$$1.4 \times 10^{8} \text{ gm}$$

Next, four different possible weights of organic material were considered.

Case I. Only foodstuffs are of concern.

Weight assumed to be 15 pounds.

Case II. Only fuel is of concern.

Weight assumed to be 460 pounds

Case III Foodstuffs and fuel of concern.

Weight assumed to be 475 pounds.

Case IV. One-thousand pounds of organic material to be released on the lunar surface

Figure 2 shows the number of grams of organic material per gram crater debris for each of these cases.

	Grams organic material/gram crater debris
Case I	4.85×10^{-5}
Case II	1.49×10^{-3}
Case III	1.54×10^{-3}
Case IV	3.24×10^{-3}

Figure 2

Finally, the numbers occurring in Figures 1 and 2 are combined in Figure 3 to give a rough estimate of the grams of organic material occurring in a sample whose surface area was 100 square centimeters at various distances from the point of impact for each of the four cases considered. Conservatively, all of the organic material is assumed to remain on the lunar surface.

distance (km)	Case I	Case II	Case III	Case IV
10	6.20×10^{-9}	1.90×10^{-7}	1.97×10^{-7}	5.80×10^{-7}
20	1.10×10^{-9}	3.37×10^{-8}	3.48×10^{-8}	1.03×10^{-7}
30	3.98×10^{-10}	1.22×10^{-8}	1.26×10^{-8}	3.72×10^{-8}
40	1.94×10^{-10}	5.96×10^{-9}	6.16×10^{-9}	1.82×10^{-8}
50	1.11×10^{-10}	3.41×10^{-9}	3.53×10^{-9}	1.04×10^{-8}
60	7.04×10^{-11}	2.16×10^{-9}	2.23×10^{-9}	6.59×10^{-9}
70	4.80×10^{-11}	1.47×10^{-9}	1.52×10^{-9}	4.49×10^{-9}
80	3.43×10^{-11}	1.05×10^{-9}	1.09×10^{-9}	3.21×10^{-9}
90	2.55×10^{-11}	7.85×10^{-10}	8.11×10^{-10}	2.39×10^{-9}
100	1.97×10^{-11}	6.05×10^{-10}	6.25×10^{-10}	1.84×10^{-9}

Figure 3 - Organic Density

If samples are 100 cm^2 exposed surface area and 10^{-8} grams is assumed to be the limit of detectability of organic material, then the dark lines in Figure 3 indicate the approximate distances beyond which organic material will not be discovered. These distances are probably high by a considerable factor due to the several "conservative" assumptions made in deriving them. In particular, the density of actual fragments of solid organic material will decrease much more rapidly with distance, and the density of organic material capable of vaporizing will be lower initially (much will move directly into space after impact) and will also be lower long term(vaporization taking place after deposition on the surface). In any event,

the maximum safe distance of about 50 km is less than the conservative safe distance (60 km) derived for biological material in the report referenced earlier.

Bioburden Experimentation and Modeling

A. Description. Models for the estimation of spacecraft bioburdens are needed for use in "clean" and "dirty" areas associated with NASA assembly environments. One such model has been developed and is described in SC-RR-69-23, "The Determination of Quantitative Microbial Sampling Requirements for Apollo Modules". An experimental program is in progress which should yield data for verification of this model and guide parameter selection for future models.

The general problem of estimating the number of microbes on an object in a clean room must be solved subject to the constraints that only certain surfaces of the object can be directly sampled and that these surfaces can be sampled only at specific times. Selected environmental measurement such as number of microbes per cubic meter or number of particles per cubic meter can be made. Subject to these constraints, there are two basic problems - 1) knowing what is on the sampled surfaces, how do we predict what is on inaccessible surfaces and 2) knowing what is on the object at a particular time, how do we predict what is on the object at a later time. The general problem is reduced to the specific ones by attempting to understand how microorganisms get on an object in a clean room by deposition from airflow and by deposition from mechanical contact; and how microorganisms are removed from an object in a clean room by airflow, by vibration, by death, and by mechanical contact. Using such knowledge, the validity of the model

referenced above may be investigated. Should the model prove reasonable the first of the two basic problems is solvable and a conceptually straightforward extension of the model can be used to solve the second. With the experimentation in progress we are addressing the problem of deposition of microorganisms by airflow.

Using the vertical laminar downflow research facility described in QR 11, a number of experiments have been performed to elucidate the effects of various parameters on the particle collection efficiency of an object exposed to an aerosol. This was done using flow conditions approximating those in a laminar flow clean room.

B. Progress

1. Collection Efficiency of a 1" x 1" Square Glass Plate

The reference value in the experiments furnishing the data for Figures 3-6 in QR 13 was obtained by placing a 1"x1"glass plate in the aerosol chamber with the test surfaces. All of the values in these three curves were referenced to the collection efficiency of this 1"x1" horizontal glass plate. Consequently, to complete the analysis of QR 13, the collection efficiency of the 1"x1" glass plate was measured accurately.

To perform this measurement, a membrane filter holder was adapted to make an isokinetic aerosol sampler. A thin-walled 3" long piece of copper tubing with a sharp leading edge was attached

to the filter holder. The flow rate was adjusted to give a flow velocity of 80 ft/min. within the tube. This velocity agreed with the hot-wire anemometer measurement of the free stream air velocity at the sample point in the aerosol chamber.

The collection efficiency was determined by sampling an aerosol simultaneously with the flat glass plate and the device described above. The two devices were placed far enough apart so as not to distrub the airflow of each other. The collection efficiency is the ratio (number of particles on the plate)/(number of particles on the filter) corrected to corresponding volumetric flow rates. A total of five separate experiments were performed with a mean collection efficiency of 14.8%.

Consequently, the ordinates labeled 1.0 (Figures 3,5,6) in QR 13 can be assigned the numerical value of 14.8%.

2. Frequency Distribution of the Number of Microorganisms per Particle in Given Size Ranges of Particles

In order to test basic assumptions that have gone into the bioburden model (SC-RR-69-23), instrumentation has been developed and tested to measure the number of microorganisms per particle for given size ranges. The hypothesis that the samples taken are from a Poisson distribution can also be tested.

The instrument that has been developed and tested is a Royco particle counter placed directly in series with an Andersen sampler. The Royco counts and sizes all particles entering the

instrument and the Andersen sampler sizes and enumerates all microbe-bearing particles. After sampling, the Andersen plates are aseptically removed in a clean room and sliced in half with a sterile knife. One-half of the agar is sonicated and the other half is allowed to grow undisturbed. The water in which the agar is sonicated, is plated out using standard techniques.

The agar that has been sonicated is then overlayed and grown out to make sure that all microorganism-bearing particles are removed from the agar by sonication.

This procedure allows three parameters to be measured:

1. the total number of particles in a given size range is determined by the Royco
2. the total number of microorganism-bearing particles in a given size range is determined by the untreated half of the Andersen plate.
3. the total number of microorganisms riding on a given size range of particles is determined by the number of colonies arising from the sonicated half of the Andersen plate.

Tests with Bacillus subtilis var. niger and Mucor spores indicated that the instrument was working correctly in that the spores were being deposited on the correct stages of the Andersen sampler. Bacillus subtilis var. niger spores (1 micron by 1.5 micron) were deposited on stages 5 and 6; and Mucor spores (5 micron spherical) were deposited on stage 3. In

testing the sonication procedure, it was found that 95% of the Bacillus subtilis var. niger spores were removed by sonication for 5 minutes in deionized water.

Preliminary tests on normal samples from our non-clean room laboratory area indicate that there are approximately 3 microbe carrying particles per cubic foot of air.

The results obtained from this experimentation will be used as indicated in the following section on Spacecraft Sampling to verify an existing static model and a dynamic model being developed both of which account for the presence of more than one microorganism per particle. It has been previously determined that a small percentage of the microbe-carrying particles carry most of the microorganisms in an air or surface sample. When one microorganism is found, the probability is increased that another will be found in the same sample. As a result of models of this phenomenon, confidence limits on the estimate of the number of organisms on a spacecraft will be different from those normally obtained. These limits can differ drastically, depending on the values of the frequency distribution of the number of microorganisms per particle.

When the procedures for the use of the instrument are fully tested, the instrument will be sent to Cape Kennedy to measure parameters in the actual field environment that is being modeled.

Spacecraft Sampling

A. Description. The objective of this activity is to obtain an estimate of the number of contractor taken biological assay samples needed for predicting the bioburden of Viking '73 at various confidence levels, and the number of samples required by planetary quarantine personnel to "verify" burden estimates made by the contractor.

B. Progress. Rough estimates of both the aforementioned numbers of samples were made this quarter. The model developed in Sandia Laboratories Research Report, SC-RR-69-23 entitled, "The Determination of Quantitative Microbial Sampling Requirements for Apollo Modules" was used to estimate the number of contractor samples required to estimate the expected burden of Viking '73 at a given sample time. Before presenting the results, a few words about this model are in order.

The model is essentially a modified "birth and death" model which allows for the possibility that organisms may be deposited on surfaces in "clumps" and removed in the same fashion. This differs from standard birth and death models which assume that two or more organisms could not be deposited on or removed from a surface at the same time. Clumping is a well known phenomenon. On the other hand, the distribution of the number of organisms per intramural particle or "clump" is not well known. In developing the surface burden model referenced above, this distribution was assumed to be Poisson. Physical experimentation described elsewhere in this quarterly

report is being undertaken to examine this assumption. In the model development, the "clumps" were subsequently assumed to be deposited in accordance with a birth and death distribution. Under many circumstances this is known to be a reasonable assumption, and, again, the reasonableness in highly controlled spacecraft assembly environments is being experimentally investigated. The mixing of the two distributions, organisms per particle and particles on a given surface leads to a Neyman's Contagious Distribution of Type A under steady state conditions (when the "particles" have reached a plateau value which is Poisson distributed). Using standard techniques with this "steady state" model yields the number of samples needed to estimate the expected number of organisms on a given surface at various confidence levels. Some of the parameters occurring in these calculations are: sampling efficiency (percent of organisms on surface that are removed), sample area, total surface area, the maximum microbial burden on the surface in question, and the expected number of organisms per "clump". Incidentally, the burden resulting from contact may, in this framework, be treated as "clumps" also, obviating a separate procedure for estimating contact burden.

In subsequent calculations, the following is assumed:

(i) a swab sampling procedure is used which

- is 70% efficient, and
- assays 4 square inches per sample,

(ii) the mean number of organisms per particle or "clump" is 4 based on recent PHS data for intramural contamination at Cape Kennedy

(iii) the maximum number of organisms on the spacecraft at any time is 10^6, and

(iv) samples are randomly taken.

Since the surface areas that may be of concern at given stages of Viking '73 assembly are not available, the numbers of samples needed to estimate the expected burden at various confidence levels have been determined as a function of surface area. The results are shown in Figure 1.

Confidence Level \ area (ft^2)	340	500	660	1000
0.99	233	343	452	685
0.95	135	198	261	395
0.90	94	139	183	277

Figure 1 - Approximate Number of Samples Needed to Estimate Expected Spacecraft Bioburden

There is currently a proposal that the Viking '73 contractor take approximately 220 samples per sampling period. The confidence levels for each area given above when 220 samples are taken are shown in Figure 2.

Area(ft^2)	340	500	660	1000
Confidence level	.993	.980	.963	.926

Figure 2 - Confidence Levels as a Function of Area for Contractor Bioburden Estimate with Approximately 220 samples

Generally speaking, the role of the Planetary Quarantine Office is assumed to be that of monitor. Rather than estimate the Viking '73 burden independently, it is assumed that the Planetary Quarantine Office wishes only to confidently verify the "hypothesis" that the contractor estimate is "reasonably"close to the actual burden. For this a different model is necessary. A standard hypothesis testing model was used, and in addition to the foregoing assumptions, the following is assumed:

(i) the hypothesis to be tested in monitoring is whether the actual mean burden, μ, exceeds the contractor estimate μ_c by more than $0.9\ \mu_c$. Notationally,

$$H: \quad \mu \geq 1.9\mu_c.$$

(ii) if μ and μ_c are actually equal, the above hypothesis should be rejected 0.995 percent of the time, and

(iii)the minimum expected bioburden of the spacecraft is one organism per square inch of surface area.

This latter assumption makes the number of samples needed to test the hypothesis(that the actual mean bioburden exceeds 1.9 times the contractor estimate)independent of the area sampled. Figure 3 gives these sample numbers as a function of the "confidence level" (one minus the percentage of time the hypothesis, even though true, is rejected).

Confidence Level	Number of Samples
0.99	57
0.95	40
0.925	39
0.90	36
0.85	32

Figure 3 - Approximate Number of Samples Needed by Planetary Quarantine Office to Determine Accuracy of Contractor Bioburden Estimates

It should be emphasized that the numbers reported here are quite approximate. The model governing the organism distribution has not yet been verified, and until this is done, results derived from the model can only be regarded as tentative. Also, a number of parameter values have been assumed even though experimental evidence is insufficient.

Several other tentative conclusions may be drawn. Based upon the models used:

(i) if sampling efficiency can be increased, the number of samples needed both by the contractor and the Planetary Quarantine organization will be reduced,

(ii) if sample area per sample is increased, the same conclusion holds, and

(iii)if better parameter values can be obtained through laboratory experimentation, more confidence in this approach can be gotten and, for given fixed bioburdens, fewer samples will be needed. Included in this category are such parameters as maximum and minimum bioburden, and mean number of organisms per "clump".

Additionally, it should be remarked that "transient" behavior may be more appropriate than "steady-state" behavior in highly controlled environments because the low bioburden may never reach a "plateau". This is particularly true if bioburden predictions with time are to be made in the early stages of assembly. Accurate estimation of low bioburdens require more samples than accurate estimation of denser loadings when a steady state approach is taken. The same will be true of a transient model, but perhaps less so. This same fact suggests that bioburden estimates of exposed surfaces be made as late in assembly as possible while sampling to estimate burdens on surfaces to be mated should come as near to the time of mating as possible. Thus, while there is not sufficient information to accurately gauge the adequacy of a sampling program with time, these observations might be tentatively used as basic guidelines.

Thermo-Radiation Sterilization (USAEC Funded)

A. Description. The thermal degradation of spacecraft components as a result of present dry heat sterilization is a problem still not completely resolved. Silver-zinc batteries, high value mylar capacitors, vidicon tubes, PBAA solid propellants, culture media, photometers and other scientific instruments suffer from high temperature environments. The purpose of the thermo-radiation study is to explore possible techniques that will reduce the dry heat time and temperature requirements for sterilization of spacecraft hardware. More specifically, the study is concerned with determining the synergistic effects of the combined application of heat and radiation.

B. Progress. The work this quarter was directed primarily toward determining the sensitivity of experiments to factors such as changes in initial sample populations and secondly, the sensitivity to varying relative humidity.

Experiments were performed to determine how the initial sample populations used in thermo-radiation experiments effected sterilization. The high initial populations of 10^9 were used to gain 7 logs of good data in the death curves. This practice has been discontinued in favor of the lower loading of 10^6 where less apparent protection is provided by the inoculum thickness. Comparison data on thermo-radiation effects with varied initial loading are shown in Fig. 1. The D value[1] changed from 1.6 hrs. with 1.4×10^9 initial loading

[1] A D value is the time to reduce a given microbial population by 90% or one log in count at a given temperature. Used here for convenience, it is not a completely apt descriptor of the survivor curves many of which were concave in character in the ranges of one to 100 survivors.

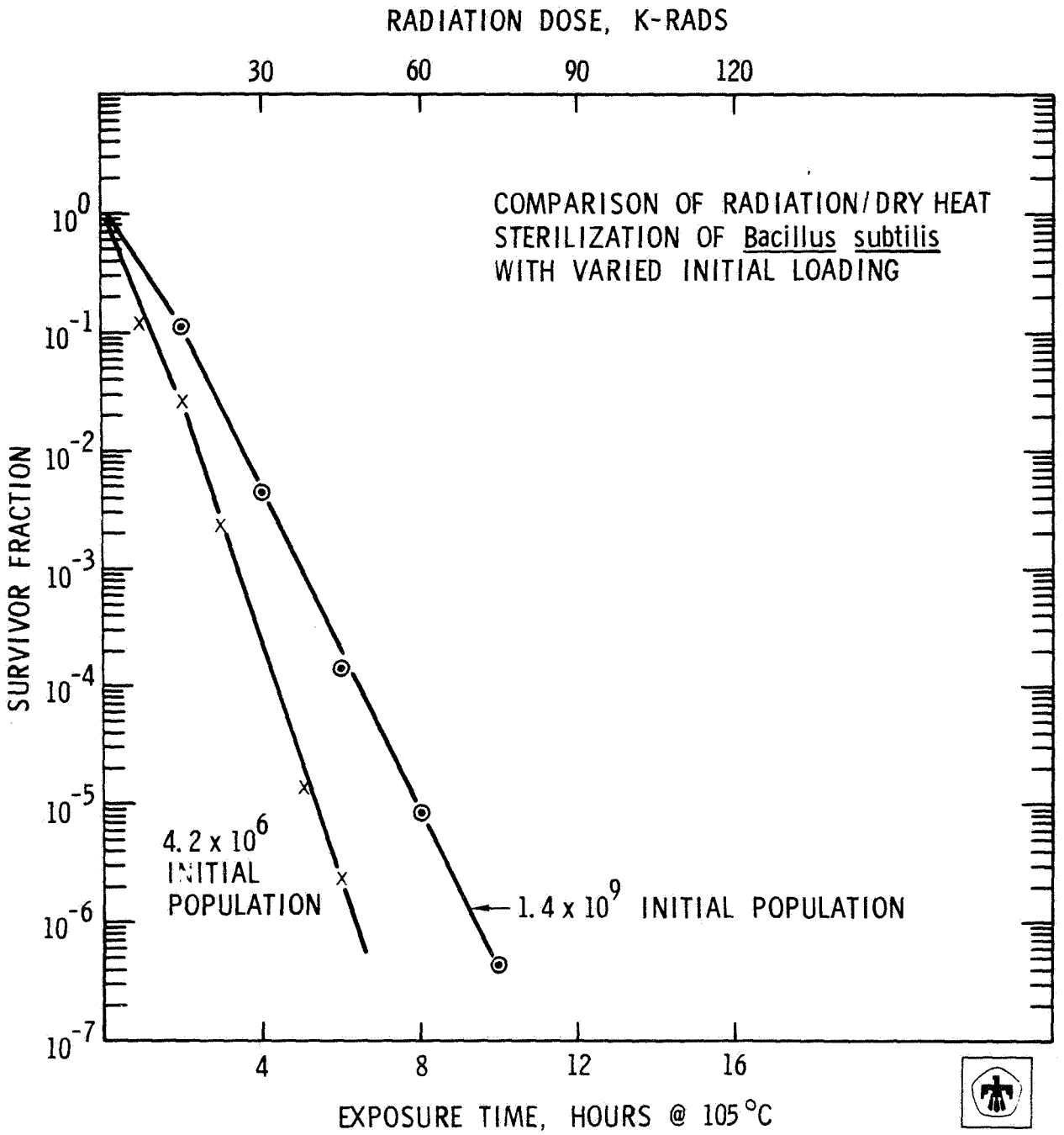
RADIATION DOSE, K-RADS
30
60
90
120
COMPARISON OF RADIATION/DRY HEAT
STERILIZATION OF Bacillus subtilis
WITH VARIED INITIAL LOADING
SURVIVOR FRACTION
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
4.2×10^{6}
INITIAL
POPULATION
1.4×10^{9} INITIAL POPULATION
4
8
12
16
EXPOSURE TIME, HOURS @ 105 °C

FIGURE I

of 4.2×10^6 B. subtilis spores/sample.

It has been known for some time that variable relative humidity affects the thermal destruction of microorganisms.[2] In some cases the "D" value changed by a factor of 10 or more as the RH was varied. Our interest in variable RH was to determine the sensitivity of thermo-radiation experiments under changing RH over a span typical of laboratory conditions, i.e., roughly 20% to 40% RH. Figure 2 shows that room air over a nominal range of 20% to 60% RH represents a range of only 0.5% to 1.5% RH after that air is elevated to an oven temperature of $100^{o}C$.

To study the variable RH effects, a system was assembled by which precise moisture control in the oven could be maintained. Figure 3 shows the system used. Room air is saturated at room temperature in one leg of the conditioning system, and dessicated in the other leg. The two flows are then mixed to the required proportions for desired RH at room temperature. The conditioned supply to the oven is controlled at a flow of 0.5 CFM. Narrow range lithium chloride sensors with an accuracy of $\pm$ 2% are used in the supply to the oven. A sample is taken from the oven at a flow of 200 cc/min., cooled to room temperature, and then measured with LiCl sensors. The sensors and printout recorder were calibrated as a system in the Primary Standards laboratory.

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[2] W. G. Murrel and W. J. Scott, "The Heat Resistance of Bacterial Spores at Various Water Activities". J. Gen. Microbiology (1966) 43, 411-425.

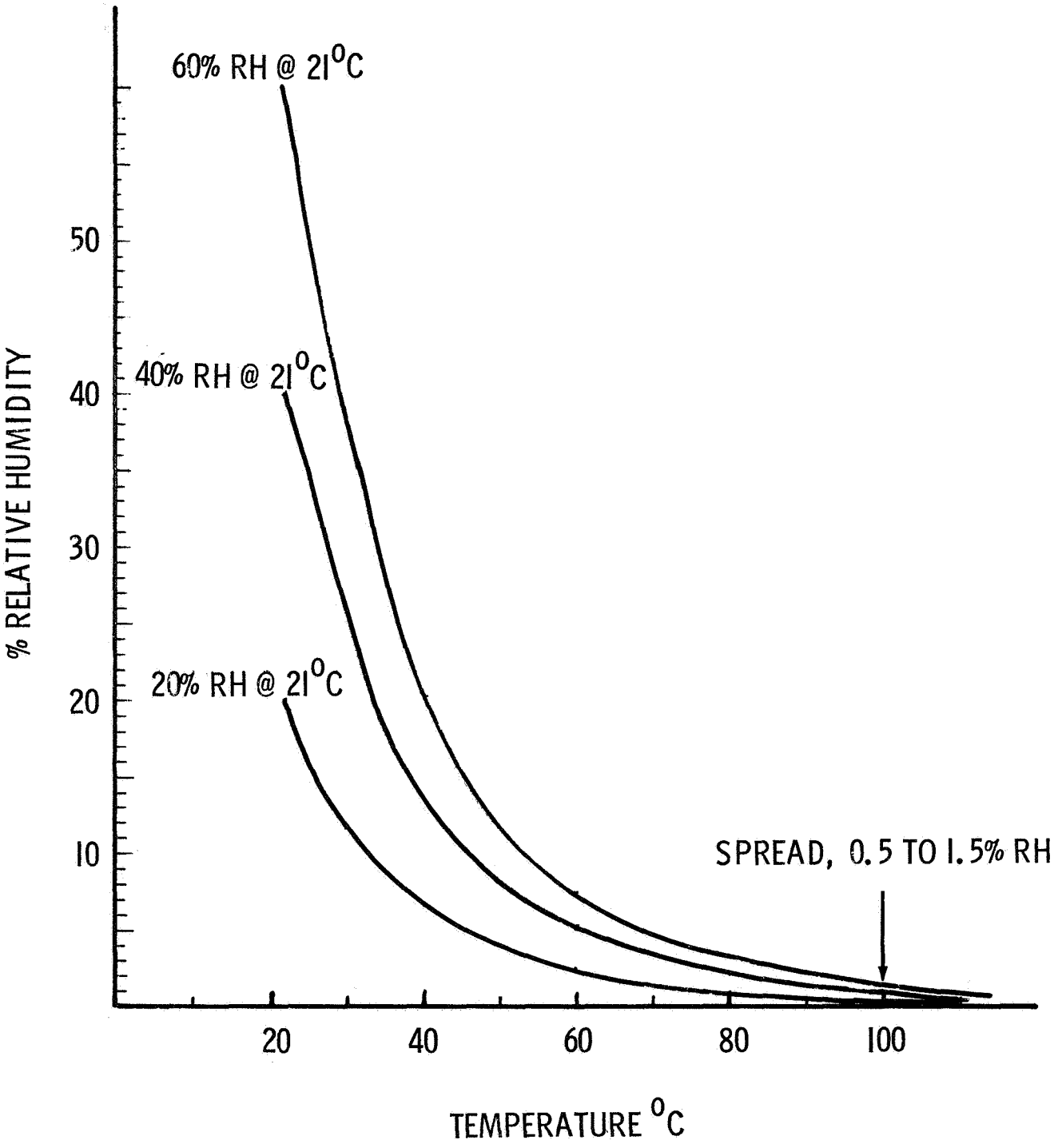
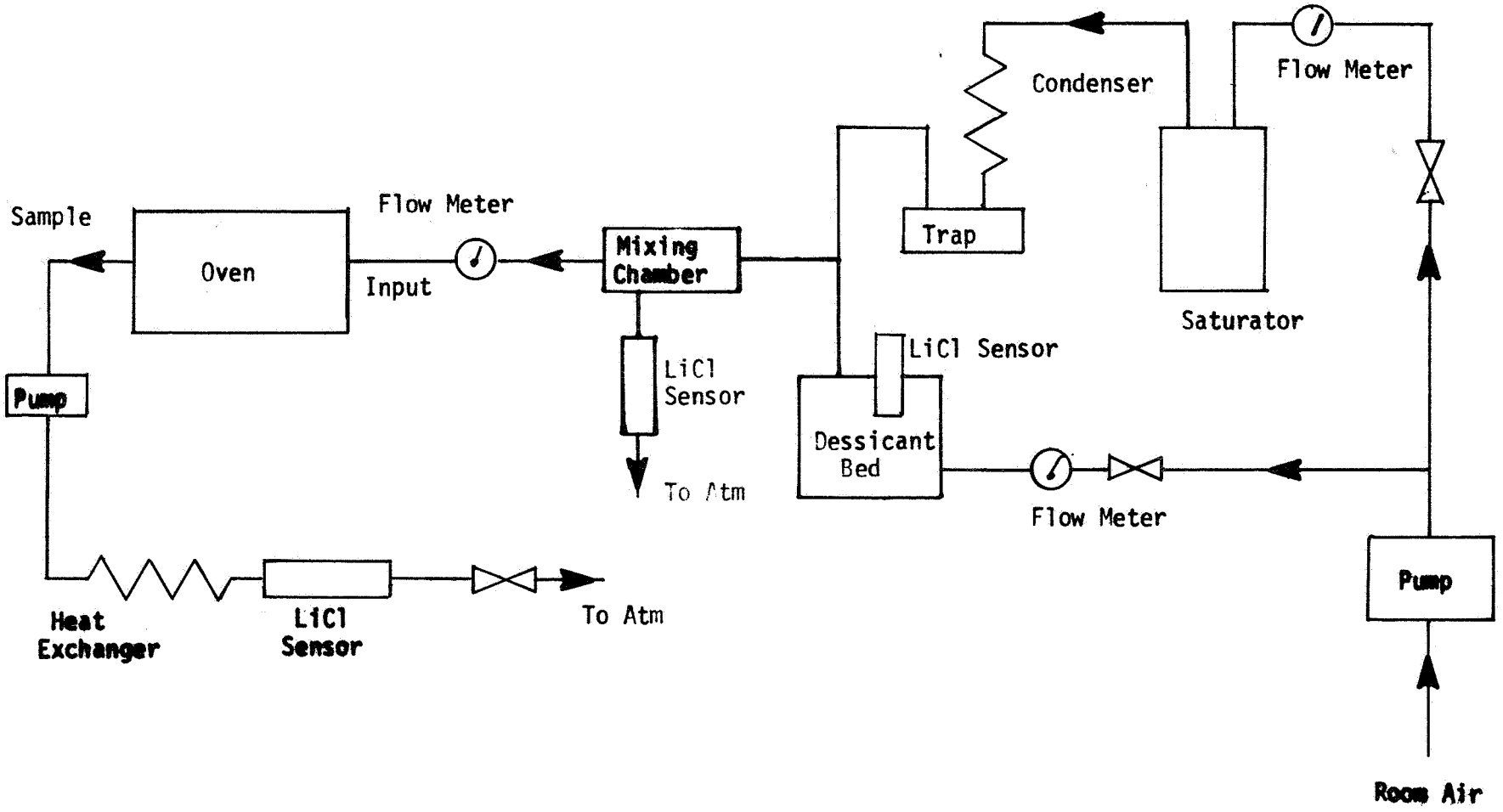

60% RH @ 21°C
40% RH @ 21°C
20% RH @ 21°C
SPREAD, 0.5 TO 1.5% RH
% RELATIVE HUMIDITY
50
40
30
20
10
20
40
60
80
100
TEMPERATURE °C

FIGURE 2



HUMIDITY CONTROL SCHEMATIC

FIGURE 3

Using the system described above, variable RH experiments were first performed with dry heat alone at 105°C to gather baseline data at room RH levels of 20%, 40% and 60%. These data were needed for design of thermo-radiation RH sensitivity experiments. Results of dry heat with variable RH are shown in Figure 4. D values ranged from 2.8 hours at 20% RH to 5.1 hours at 60% RH. Conditions in the Sandia laboratory would normally range from 20% RH to 40% RH with a D value variation from 2.8 hours to 3.5 hours.

The same levels of RH, i.e., 20%, 40% and 60% were used in thermo-radiation experiments, the results of which are shown in Figure 5. It was interesting to find that the variable RH seems to have little effect on the survivor curves. This might be explained by noting the opposing effects water can have on the lethality of heat as compared to its effects with radiation. Figure 4 illustrated the increase in heat resistance of B. subtilis over a range of 20% to 60% RH at room temperature (0.5% to 1.5% RH in the over). A number of authors[3,4,5] have noted a decrease in the radiation resistance of organisms as the moisture content these ranges was increased. This suggests that heat and radiation when used simultaneously in the range of 20%-60% RH room ambient appear to lose the annoying sensitivity to varying RH conditions.

---

[3] G. Sykes,"Disinfection and Sterilization", Sec. Edit.1967,J.P.Lippincott Co.

[4] G.E.Stapleton and Alexander Hollender,"Mechanism of Lethal and Mutagenic Action of Ionizing Radiations on Aspergillus Terreus", J.Cellular Comp. Physiol. 39, Suppl. 1, 101-115.

[5] A.Tallentire, H.A.Dickson and J.H.Collett,"A Dependence on Water Content of Bactericidal Efficiency of Gamma Radiation", J. Pharm Pharmacol. 15: Suppl. 180-1, Dec. 1963.

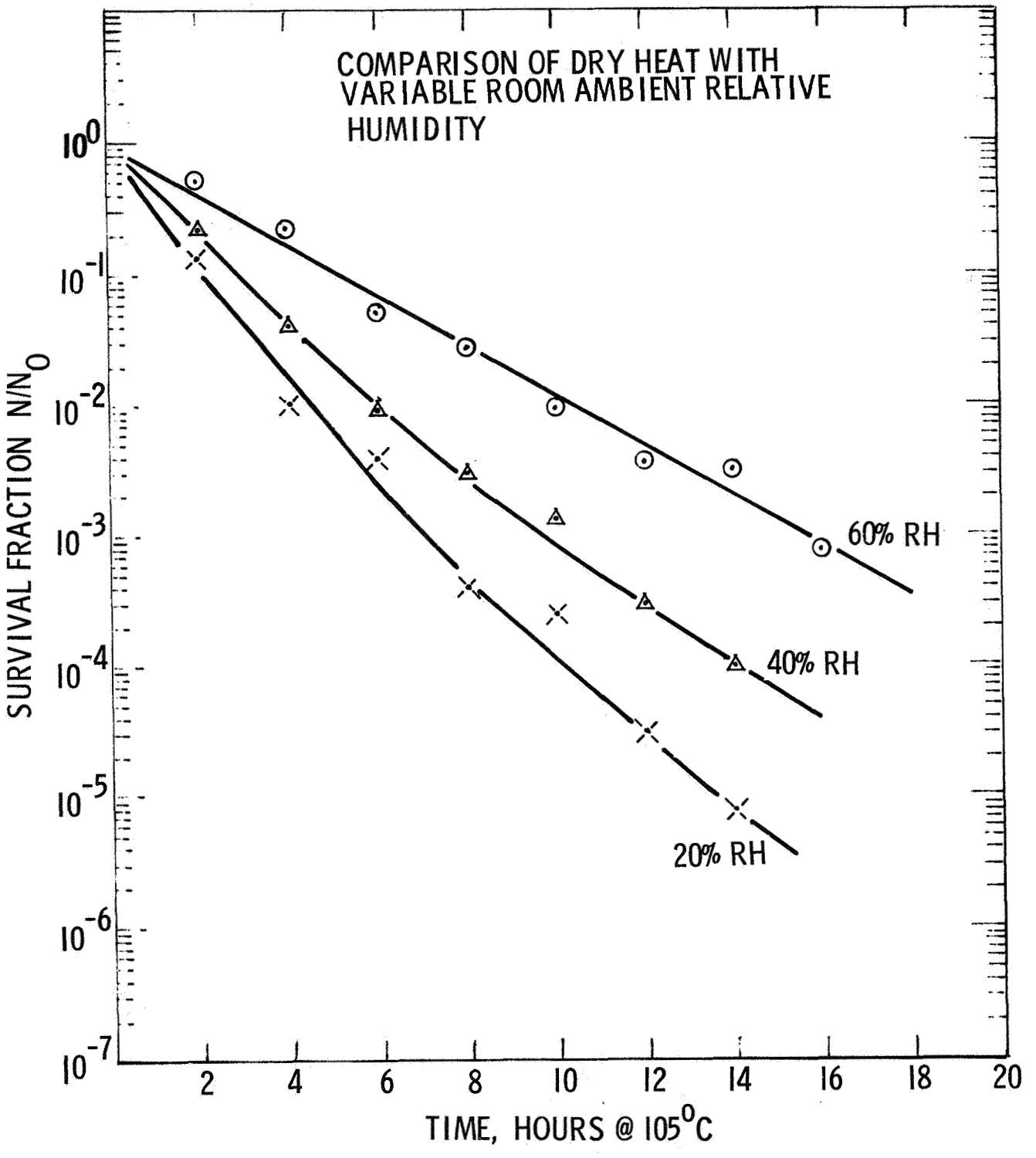

COMPARISON OF DRY HEAT WITH
VARIABLE ROOM AMBIENT RELATIVE
HUMIDITY
SURVIVAL FRACTION N/N0
10^0
10^-1
10^-2
10^-3
10^-4
10^-5
10^-6
10^-7
60% RH
40% RH
20% RH
2
4
6
8
10
12
14
16
18
20
TIME, HOURS @ 105°C

FIGURE 4

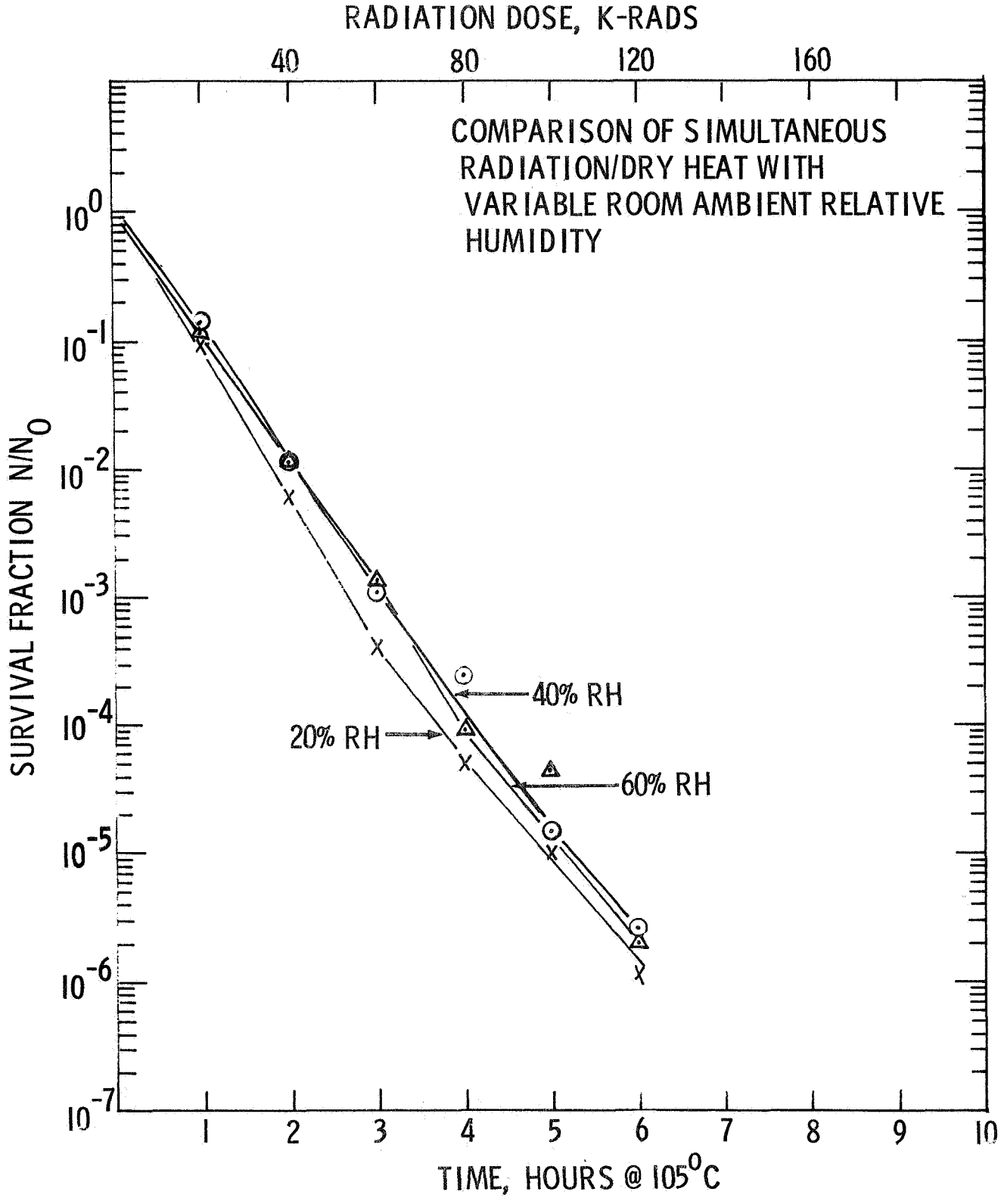

RADIATION DOSE, K-RADS
40
80
120
160
COMPARISON OF SIMULTANEOUS RADIATION/DRY HEAT WITH VARIABLE ROOM AMBIENT RELATIVE HUMIDITY
SURVIVAL FRACTION N/N0
10^0
10^-1
10^-2
10^-3
10^-4
10^-5
10^-6
10^-7
40% RH
20% RH
60% RH
1
2
3
4
5
6
7
8
9
10
TIME, HOURS @ 105°C

FIGURE 5

Federal Standard 209a

A. Description. The General Services Administration (GSA) requested that Sandia Laboratories review Federal Standard 209a "Clean Room and Work Station Requirements, Controlled Environment" and determine whether the standard should be revised.

B. Progress. Federal Standard 209a was thoroughly reviewed and several types of users were contacted to determine the status of the current issue, which was last revised in 1966. The results of this study and the concensus of the users may be summarized as follows:

1. The basic technical data regarding the definition of air cleanliness classes is sound and should not be changed. This subject represents a major portion of the mandatory section.
2. Some confusion and misunderstanding exists in two areas, i.e., (a) the relationship between and proper use of the mandatory and nonmandatory sections of the standard, and (b) the proper techniques for testing and monitoring to determine compliance with the standard.
3. The standard contains several typographical and minor numerical errors and numerous instances in which the wording and composition do not convey the intended meaning in the most succinct manner.

It is evident that some changes are desirable, but there appears to be no urgent need for technical changes. Sandia Laboratories informed GSA of this conclusion and recommended that a meeting of interested government agency representatives be called for the fall of 1970 to reconsider the need to revise the standard.

Presentations:

1. H. D. Sivinski, presented two papers entitled, "Kinetic Model of Bacterial Inactivation" and "Fine Particle and Aerosol Physics Studies", at the AIBS NASA Symposium on Spacecraft Sterilization Technology, Las Vegas, Nevada, September 24,25, 1969.

2. M. C. Reynolds, presented a paper entitled, "Thermo-Radiation Studies", at the AIBS NASA Symposium on Spacecraft Sterilization Technology, Las Vegas, Nevada, September 24,25, 1969.

3. A. L. Roark, presented paper entitled, "Lunar Planetary Quarantine Systems Study and Information System", at the AIBS NASA Symposium on Spacecraft Sterilization Technology, Las Vegas, Nevada, September 24,25, 1969.

4. C. A. Trauth, Jr., presented a paper entitled, "Predicting Bioburdens in Controlled Environments", at the AIBS NASA Symposium on Spacecraft Sterilization Technology, Las Vegas, Nevada, September 24,25, 1969.

5. H. D. Sivinski, presented paper at Southwest Regional Conference of AACC Meeting in Dallas, Texas, September 9, 1969.

6. W. J. Whitfield presented paper entitled "Clean Room Environment" and was a member of the faculty at the Principles and Practices of Contamination Control Conference, University of Alabama, Huntsville, Alabama, August 18-22, 1969.

7. C. A. Trauth, Jr., presented paper entitled, "A Systems Approach to Contamination Control", at a Seminar on Principles of Contamination Control, University of Alabama, Huntsville, Alabama, August 18-22,1969.

Miscellaneous:

1. H. D. Sivinski attended the International Academy of Astronautics Orbiting Laboratory and Space Science Conference, Cloudcroft, New Mexico, September 28-October 2, 1969.

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